Ischemic Heart Disease and Platelet Aggregation

The Caerphilly Collaborative Heart Disease Study

Peter C. Elwood, MD, FRCP; Serge Renaud, PhD; Dan S. Sharp, MD, PhD; Andrew D. Beswick, BSc; John R. O’Brien, DM, FRCP; and John W.G. Yarnell, MD, MFCM

The Caerphilly Collaborative Heart Disease Study is based on a large cohort of men (2,398) aged 49–66 years at the time of study. Platelet aggregation induced by collagen, thrombin, and ADP was measured in fasting blood samples and was related to prevalent angina, past myocardial infarction, and electrocardiographic evidence of ischemic heart disease. A number of subjects had taken aspirin, other nonsteroidal anti-inflammatory drugs, or other drugs affecting platelet aggregation 7 days before blood sample collection; after the exclusion of these subjects, data were available for 1,811 men. No relations were demonstrated with angina, but significant relations were shown between past myocardial infarctions and electrocardiographic evidence of ischemia and ADP-induced aggregation (both primary and secondary) and between electrocardiographic evidence of ischemia and thrombin-induced aggregation. The strongest relation indicated more than a twofold increase in the odds of a past myocardial infarction in subjects of the highest fifth of ADP-induced primary platelet aggregation compared with the lowest fifth. No significant relations were detected with collagen-induced aggregation. Accounting for a number of possible confounding factors had a relatively small impact on the relations between platelet aggregation and ischemic heart disease. Other evidence, including the well-established effect of aspirin on reducing the incidence of ischemic heart disease, indicates that the relations we describe are unlikely to be simply an effect of IHD on platelets. (Circulation 1991;83:38–44)

The role of hemostasis in ischemic heart disease (IHD) is a developing area of research. Attention is focusing on thrombotic mechanisms and on the role of platelets in thrombosis. Indeed, case-control studies suggest a definite relation between platelet aggregation and vascular disease.1,2 Postmortem studies have demonstrated an involvement of platelets in coronary artery thrombi,3,4 and platelet emboli have been detected in the coronary microcirculation in sudden death.5 Angiographic observations have directly demonstrated the thrombotic occlusion in acute myocardial infarction (MI).6 The diurnal pattern of infarction has been associated with platelet aggregability,7 and aggregation has been shown to be enhanced in the presence of factors relevant to an increased risk of infarction, such as a high intake of saturated fat8 and diabetes.9 Further evidence comes from animal studies in which, for example, platelet aggregates have been shown to cause a reduction in blood flow when stenosis is produced experimentally in a coronary vessel.10 However, the marked reduction of coronary events by aspirin constitutes the most convincing evidence of the role of platelets in IHD.11–13 The main effect of aspirin in this context is probably an inhibition of platelet aggregation, and this effect appears to be reflected best in the response of platelets to ADP and to collagen.

However, there has been no direct demonstration that platelet reactivity is relevant to IHD in the general population. The only relevant study so far is by Meade et al.,14 but this study was based on only 635 white males older than 25 years, of whom only 78 had evidence of IHD (24 with angina or a history of infarction and 54 with electrocardiographic evidence of ischemia). In this report, we present evidence on the relevance of platelets to IHD in more than 2,000 men involved in the Caerphilly Collaborative Heart Disease Study,15 of whom 226 had a history of a past infarction and 380 had electrocardiographic evidence of ischemia.
Methods

The total cohort at the time of examination comprised 2,398 subjects aged 49–66 years, of whom 2,176 (90.7%) had platelet aggregation tests. Two hundred twenty-two subjects did not submit to venu-puncture but did complete questionnaires, did undergo electrocardiography, and have their blood pressures measured. There was no significant difference in the proportion of subjects 1) with prevalent angina, past MI, or electrocardiographic evidence of IHD, 2) taking medication that affected platelet function, or 3) among smoking status categories who did and did not have platelet aggregation tests. Subjects having platelet tests were slightly older (57.5 versus 56.5 years) but demonstrated no significant difference in average values of systolic or diastolic blood pressure or body mass index (kg/m²).

Three indicators of prevalent IHD were assessed: past MI, electrocardiographic evidence of ischemia, and angina. The categorization is described in detail in a previous report16 but briefly described herein.

The London School of Hygiene and Tropical Medicine chest pain questionnaire was completed by all subjects. A positive angina history was denoted by grade 1 or grade 2 categorization from the anginal portion of the questionnaire. Past MI required both a positive response to the question about severe chest pain for at least 30 minutes and an indication of a physician’s diagnosis of an ischemic event. Electrocardiographic evidence grouped probable ischemia (Minnesota Codes 1-1 and 1-2) and possible ischemia (codes 1-3, 4-1 to 4-4, 5-1 to 5-3, and 7-1) as positive evidence.

Men attended an early morning clinic to give a fasting blood sample. The first 30 ml blood was used for nonplatelet tests, and then blood was drawn without stasis into 0.13 M sodium citrate anticoagulant (one part per nine parts blood). Platelet-rich plasma (PRP) for platelet aggregation tests was prepared within 10 minutes of venipuncture exactly as described by Renaud et al.8,17 A mobile laboratory specifically designed for population studies was used.

Platelet activity was measured in PRP by turbidometric methods originally described by Born and O’Brien19 and adapted by Renaud et al.18 for epidemiological studies. The instrument (Rubel-Renaud coagulooagregometer) was designed to have response characteristics that can be precisely and routinely assessed and readjusted if necessary to be certain of obtaining the same optical density response during a period of several years. Also, its sensitivity is better than that of commercial aggregometers.

The extent of aggregation in duplicate to single doses of three reagents was measured in PRP adjusted to 300,000 platelets/µl with autologous platelet-poor plasma (PPP).8,17 The reagents were collagen (soluble skin collagen, Worthington Diagnostic Systems Inc., Freehold, N.J., 42.7 µg/ml in PRP), adenosine diphosphate (Sigma Chemical Co., St. Louis, Mo., 0.725 µM/l in PRP), and thrombin (Sigma Chemical Co., 0.056 units/ml in PRP). Measurements were limited to assessment of the optical density response to a single dose of reagent for logistic reasons in the belief that a single measurement on a large number of subjects would lead to more precise estimates of relation than would multiple measurements on a small number of subjects. An assessment of platelet aggregation on the entire cohort is demanded because the data will ultimately be used to establish relations with future, but unknown, incident events.

For all tests, the maximum optical density increase due to platelet aggregation was measured and expressed as a proportion of the difference in optical density between PRP and PPP. For collagen, the proportionate change in optical density was measured after 7.5 minutes. In the case of ADP, the response of the platelets is biphasic, a primary wave of aggregation occurring as a direct response to the added ADP and then a secondary wave in response to ADP and other agonists liberated by the platelets themselves. Both these responses (primary and secondary) were measured. The secondary response was measured as the extent of optical density change from baseline at 2–2.5 minutes after addition of ADP. In contrast to thrombin-induced aggregation, this change never regained the baseline, and in 1,681 subjects, the measure was less than that for the primary response. This failure to regain baseline was considered to be a quantitative estimate of irreversible aggregation regardless of whether the optical density measurement was greater or less than that for the primary response.

This measurement of ADP-induced secondary aggregation is highly operational but not inconsistent with the idea of a thromboxane-mediated “irreversible” effect of aggregation induced by ADP. The validity of this operational definition was borne out by a demonstrated relation with the use of medications affecting platelet aggregation, but no relation with ADP-induced primary aggregation in which there is postulated no involvement of cyclooxygenase mechanisms (see “Results”).

The indexes of aggregation that are presented here are, therefore, for each agonist separately: “Aggregation” is equal to optical density change with agonist divided by maximum possible change (i.e., PPP).

Because of the large number of subjects and the extended period of data collection, new batches of reagents had to be prepared from time to time. Every new batch was tested against the previous batch. Unfortunately, in the course of the study, Sigma Chemical Co. changed the technique to evaluate the reactivity of thrombin. Thus, a major readjustment had to be made in the concentration of thrombin used for tests in order to match reactivity before the change.

Despite the readjustment, a statistically significant difference in mean levels of aggregation was still noted before and after the change and was attributed solely to this methodological alteration. Therefore,
measurements using the old thrombin reagent were rescaled to the mean and standard deviation of the measurements using the new reagent by a linear transformation\textsuperscript{20} before relations with IHD categories were examined. Adjustments associated with batch-to-batch variation in the preparation of ADP reagent were also done for ADP-induced primary aggregation. Means and standard deviations within a batch were rescaled to the grand mean and pooled-batch standard deviation, but these adjustments were much smaller in magnitude than those done for thrombin. Such adjustments attribute a proportion of interindividual variation to differences between batches and, thus, accounts for a degree of measurement imprecision.

Preliminary studies on this population had shown that the standard error of a single estimate within subjects was less than 5\% of the range of mean values between subjects.\textsuperscript{21} Thus, measurements of platelet aggregation in PRP at a single dose of agonist can be used to estimate measures of association without fear of substantial biasing.\textsuperscript{22}

Relations between IHD categories and the PRP platelet aggregation tests were assessed using logistic regression methods to model relative odds ratios. Assessment of confounding as the cause of relations between platelet tests and IHD is important in observational studies such as this one. The effect of adjustment for potential confounders such as serum cholesterol (total and high density lipoprotein), smoking status (never smoked, four levels of former smokers based on years since cessation, and four levels of current smokers based on 1–14, 15–24, and more than 25 cigarettes per day, and exclusive use of cigars or pipes, which was dummy coded relative to the never smoked status), systolic and diastolic blood pressures, body mass index (kg/m\(^2\)), and age were examined by noting how the inclusion of these co-

<table>
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<th>TABLE 1. Proportion of Men by Age Group in the Caerphilly Cohort With Ischemic Heart Disease</th>
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<td>Age (yr)</td>
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<td>----------</td>
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<tr>
<td>&lt;55</td>
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<tr>
<td>55–59</td>
</tr>
<tr>
<td>60+</td>
</tr>
<tr>
<td>Total</td>
</tr>
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Data are incomplete for a few subjects.
MI, myocardial infarction; ECG IHD, electrocardiographic evidence of ischemic heart disease.

The distributions of aggregation induced by thrombin and primary aggregation induced by ADP were unimodal and slightly skewed for thrombin. Table 3 depicts means, standard deviations, medians, and ranges for these two tests. Their distributions were reduced to groupings of "fifths" for subsequent statistical analyses.
(2,111) for whom there were complete data on all measures. Correlations between variables measured on a continuous scale (e.g., primary ADP and thrombin) are Pearson correlation coefficients, between continuous and dichotomous variables (e.g., thrombin and collagen) are biserial correlations, and between two dichotomous variables (e.g., collagen and secondary ADP) are Phi correlations. Correlations between the platelet tests and cholesterol measures are all less than 0.044 in magnitude and not statistically significant at p<0.05 in all but one case.

Relations between each test of aggregation and use of nonsteroidal anti-inflammatory drugs, including aspirin, or other drugs affecting platelet aggregation (e.g., dipyridamole) during the week before specimen collection were examined. In the case of collagen and secondary aggregation to ADP, the odds ratios of having "low" aggregation when taking such medications relative to the odds ratio of "low" aggregation when not taking medications were 1.86 (p<0.001) and 1.89 (p<0.001), respectively. There were slight, but insignificant, decreases in the average extent of aggregation in medication users for the other two tests; these being 0.1040 versus 0.1044 (p=0.91) and 0.2494 versus 0.2565 (p=0.15) for aggregation induced by thrombin and primary aggregation induced by ADP, respectively. Because of these relations, all further analyses were restricted to 1,811 subjects who had not taken medications that affect platelet aggregation within 1 week before undergoing the platelet tests.

No relations were noted between angina and any of the tests. Results that follow are, therefore, confined to relations for past MI and electrocardiographic evidence of ischemic disease.

The relative odds ratios of past MI or prevalent electrocardiographic evidence of IHD are presented for collagen in Table 5. There is no suggestion of a relation for either of the IHD categories.

In contrast, significantly elevated relative odds ratios are noted with secondary aggregation induced by ADP for past MI and electrocardiographic evidence of IHD (Table 5). Adjustment for potentially confounding covariates had little effect on the magnitude of the relations (past MI: OR\textsubscript{adjusted}=1.65, p<0.05; ECG IHD: OR\textsubscript{adjusted}=1.35, p=0.08).

Figure 2 depicts the trend in relative odds ratios of past MI and electrocardiographic evidence of IHD among groupings of "fifths" of the distribution of

<table>
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<th>Table 3. Mean, Standard Deviation, Median, and Range of Thrombin-Induced and Primary ADP-Induced Platelet Aggregation</th>
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<tr>
<td>( n )</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Thrombin induced</td>
</tr>
<tr>
<td>Primary ADP induced</td>
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</tbody>
</table>

**Figure 1. Bar graphs of distribution of platelet aggregation to collagen and to ADP (secondary aggregation).**

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thrombin-induced aggregation. A significant trend is noted only for electrocardiographic evidence of IHD.

Figure 2 also shows the results for primary aggregation induced by ADP. Marked and significant trends among the groupings of men by “fifths” for primary aggregation induced by ADP are noted for past MI and for electrocardiographic evidence of IHD. Changes in the relative odds ratios were then examined after the addition of potentially confounding covariates, using the originally scaled variables for thrombin and primary aggregation induced by ADP and calculating the relative odds ratio of IHD at the 90th percentile compared with the 10th percentile. For thrombin-induced aggregation and IHD determined by electrocardiographic evidence, the unadjusted odds ratio was 1.55, and the adjusted odds ratio was 1.52 (p<0.05). For primary aggregation induced by ADP and past MI, the relative odds ratios were 1.81 and 1.62, respectively (p<0.05), and for IHD determined by electrocardiographic evidence, the relative odds ratios were 1.72 and 1.57, respectively (p<0.05).

Discussion

These results are the first demonstration of significant positive relations between IHD and platelet aggregation in a major population study. Relations are shown with past MI and with electrocardiographic evidence of ischemia. These are shown most consistently with aggregation induced with ADP, less consistently with thrombin, and not at all with collagen. No significant relations are demonstrated with angina, but this may be due to misclassification of angina when assessed by questionnaire and to the fact that angina is more likely to be a consequence of atheroma rather than solely of thrombosis.

These results are not inconsistent with those of Meade et al.,14 who demonstrated a similar relation of increased platelet “sensitivity” to ADP agonists in subjects with prevalent IHD. Unfortunately, their results were not statistically significant, but this is probably due to the smaller number of subjects (635) and too few men in the older ages in their study.

The bimodality of the distributions of aggregation induced by collagen and of secondary aggregation induced by ADP is of interest (Figure 1). Such distributions are rarely found in the general population, and of itself, this observation indicates the need for more work on platelet function, its determinants, and its relations with IHD.

The four platelet tests of aggregation were moderately correlated with each other (r=0.235 to 0.480).

![Figure 2. Plots of relative odds ratios of prevalent ischemic heart disease in successive fifths of men defined by platelet aggregation induced by thrombin and by ADP (primary aggregation). There are approximately 362 subjects per fifth. p is associated with a χ² test of trend. EKG, electrocardiographic evidence; MI, myocardial infarction.](http://circ.ahajournals.org/)

**TABLE 4. Correlation Coefficients Among the Four Measures of Platelet Aggregation and Serum Cholesterol for Subjects With Complete Data on All Variables**

<table>
<thead>
<tr>
<th></th>
<th>Primary ADP</th>
<th>Thrombin</th>
<th>Secondary ADP</th>
<th>Collagen</th>
<th>Total cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin</td>
<td>0.480</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td>(&lt;0.0001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary ADP</td>
<td>0.441</td>
<td>0.238</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Collagen</td>
<td>0.357</td>
<td>0.235</td>
<td>0.431</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td></td>
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</tr>
<tr>
<td>Total cholesterol</td>
<td>0.030</td>
<td>-0.036</td>
<td>-0.021</td>
<td>-0.022</td>
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</tr>
<tr>
<td></td>
<td>(0.168)</td>
<td>(0.100)</td>
<td>(0.340)</td>
<td>(0.303)</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.044</td>
<td>0.015</td>
<td>-0.022</td>
<td>0.024</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>(0.042)</td>
<td>(0.483)</td>
<td>(0.314)</td>
<td>(0.274)</td>
<td>(0.026)</td>
</tr>
</tbody>
</table>

Values in parentheses are probabilities. Correlations between continuously scaled variables are Pearson correlations, between continuous and dichotomous variables are biserial correlations, and between dichotomous variables are Phi correlations. HDL, high density lipoprotein.
However, correlations between serum cholesterol (total and high density lipoproteins) and the platelet tests were exceedingly small and statistically significant at $p \leq 0.05$ in only one case.

The reduction of MI by aspirin is well documented. Aspirin affects mostly the thromboxane-dependent mechanisms of platelet aggregation, and such effects are demonstrated in our data in the significant decreases in secondary aggregation induced by ADP and in aggregation induced by collagen in the men who had used medications affecting platelet aggregation. It is perhaps surprising, therefore, that no statistically significant relation is noted between aggregation induced by collagen and any IHD category.

Lack of relation between medication use and primary aggregation induced by ADP or aggregation induced by thrombin is not surprising. Nonsteroidal anti-inflammatory drugs, and in particular aspirin, selectively inhibit cyclooxygenase and the subsequent production of thromboxane. Thrombin-induced platelet aggregation and the metabolic pathways associated with ADP-induced primary aggregation do not involve cyclooxygenase activity. Indeed, aspirin treatment has no effect in inhibiting aggregation induced by thrombin.

Positive relations were noted with both ADP-induced primary and secondary aggregation both for past MI and for electrocardiographic evidence of IHD. It would not be appropriate, however, to conclude that mechanisms relating to both primary and secondary aggregation induced by ADP play a role in the demonstrated relations with prevalent IHD. These two measures of platelet reactivity are relatively well correlated (biserial $r = 0.439$).

The relations with past MI and electrocardiographic evidence of IHD appear to be much stronger with ADP-induced primary aggregation compared with thrombin-induced aggregation. However, this may be an artifact due to the manner in which the measurements were obtained. The ADP measurements have a wider range of proportionate maximal extent of aggregation (mean, 0.2504; SD, 0.0775) compared with thrombin (mean, 0.1041; SD, 0.0562). This may be due to the use of a relatively weak concentration of thrombin compared with ADP in obtaining a range of responses in the population. However, this “weaker” concentration was dictated by a balance between avoiding outright clotting of the sample and eliciting a minimal response in all subjects of the population. Thus, these “weaker” associations for the IHD and thrombin relations are not necessarily evidence that mechanisms mediated by thrombin-induced aggregation are of lesser importance.

If these differences in relation with IHD between thrombin- and ADP-induced primary aggregation are not due to methodological factors, then the question of whether dietary fat may play a role in this difference is raised. An increased intake of saturated fat appears to promote thrombin-induced platelet aggregation. However, increased sensitivity to ADP-induced aggregation may be related both to increased intake of saturated fat and to increased polyunsaturates resulting in polyunsaturated to saturated fat ratios in excess of 1.0.

The relations reported are based on prevalent disease, and the increase in platelet sensitivity in the men with evidence of prevalent disease can, therefore, be a result rather than a cause of the ischemic disease process. Although more definitive answers will come from studies of incident disease in this cohort, there is already evidence, additional to that provided by the trials of aspirin prophylaxis, that suggests that changes in platelets, and more particularly changes in megakaryocytes, precede infarction. It has been shown that platelet reactivity is greater in large dense platelets, and patients who have sustained an MI have larger and more dense platelets than do control patients. Large dense platelets appear to be derived from megakaryocytes with high-ploidy numbers, and megakaryocytes with high-ploidy numbers are larger than those with low-ploidy numbers. Patients sustaining an MI or sudden cardiac arrest have larger megakaryocytes and increased frequency of high-ploidy megakaryocytes from specimens collected within 24 hours of the event compared with control patients. Because timing of specimen collection was close to the event, these
characteristics of megakaryocyte size and ploidy number are likely to have been determined well before the acute cardiac event.

Incident data relevant to platelet aggregation will be forthcoming from the Caerphilly Study in a few years. If measures of platelet aggregation follow the patterns that we report for plasma fibrinogen, plasma viscosity, and white cell count, and if the relative odds ratios for the platelet tests can be expected to be considerably greater for incident IHD than for those that we now report for prevalent IHD. In this same cohort of men, we demonstrate relative odds ratios for incident IHD events and fibrinogen, plasma viscosity, and white cell count of between 3.2 and 4.5 in the highest fifth compared with the lowest fifth, whereas the relative odds ratios for prevalent disease were between 1.9 and 2.3.

Tests of platelet function are likely to be of particular interest in relation to prophylaxis because certain aspects of platelet reactivity can be so easily modified by aspirin, and this drug has been shown to reduce IHD incidence. Furthermore, simple tests in a subset of this cohort (256 subjects) that measure ADP-induced platelet aggregation in whole blood are moderately correlated with ADP-induced primary (r=0.22, p=0.002) and strongly related to prevalent IHD (relative odds ratio for past MI, 7.3; p<0.05).

Current research suggests that hemostatic factors are an important cause of IHD independently of effects related to lipid metabolism, and the role of platelet function, in particular, may play a pivotal role in such causation.

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KEY WORDS • myocardial infarction • smoking • cholesterol • ischemic heart disease • Caerphilly Collaborative Heart Disease Study • clinical trials
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